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DRUG DISCOVERY

Evaluation of the effectiveness of the methanol leaf extract and fractions of *Glyphaea brevis* (Spreng) Monach (*Malvaceae*) used in the treatment of diarrhea in Nigeria

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ABSTRACT

Traditional healers and many local residents in Nigeria use a wide range of medicinal plants in the treatment of diarrhea. *Glyphaea brevis* is one of such plants claimed to have antidiarrheal property. This study investigated the antidiarrheal effects of crude methanol extract (CE) of *Glyphaea brevis* and its fractions [n-hexane (HF), ethyl acetate (EF), dichloromethane (DCMF), and methanol fractions(MF)] using castor oil-induced diarrhea and gastrointestinal(GI) motility models. The antimicrobial activity on organisms capable of causing infective diarrhea was also investigated. The extract and the fractions were also subjected to phytochemical analysis and acute toxicity test. Our results show that the groups that received DCMF (250mg/kg and 500 mg/kg) and CE (500mg/kg) elicited significant ($p<0.01$, $p<0.001$) reduction in the number of wet stool in a dose–dependent manner with DCMF (500mg/kg) offering the highest (94.44%) protection from castor oil-induced diarrhea. In the GI motility test, MF (250 and 500 mg/kg) significantly ($p<0.01$) inhibited the transit of charcoal meal caused by propulsive movement of gastro-intestinal tract offering 75.72% and 80.12% inhibition respectively while the other fractions produced non-significant inhibition. The extract and fractions were devoid of any antimicrobial activity on the organisms tested. The phytochemical analysis revealed the presence of tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids. The acute toxicity (LD_{50}) was above 5g/kg. This study therefore, validates the use of this plant by Nigerian traditional healers and local residents in the treatment of diarrhea.

Keywords: Antidiarrhea, Antimicrobial, Gastrointestinal motility, Charcoal meal, Castor oil-induced diarrhea, *Glyphaea brevis*

1. INTRODUCTION

Diarrhea is a disease condition in which there is a frequent passage of loose or watery stool three or more times during a 24-hour period (WHO, 2016). Diarrhea is one of the most common gastrointestinal disorders worldwide. Nearly1.7 billion cases of diarrheal disease are recorded each year (WHO, 2017). It is also the second leading cause of death in children under 5 years of age and is responsible for the death of approximately 525,000 children under five annually (UNICEF, 2016). It is postulated that diarrhea, even though preventable kills more people than measles, AIDS and malaria joined together in this age group (UNICEF, 2016).

The disease is mainly caused by contaminated food and water, both of which are common features in developing and underdeveloped countries of which Nigeria is one of them. In recent times, there has been a great interest in herbal remedies for the treatment of a number of ailments. The search for alternative remedy in the treatment of diarrhea was introduced by World Health Organization to overcome the side effects of antimotility agents like constipation, bloody stool, abdominal distension, stomach pain etc. Several plants have been reported to be useful in the management of diarrhea (Osadebe et al., 2012).

Glyphaea brevis (*G. brevis*) (*Malvaceae*) is reputed for its beneficial effects in the treatment of diarrhea in south east Nigeria. It is a spreading shrub, climber or small tree up to 8m high. It is very common in undergrowth of closed forest, secondary jungle, river banks, low lands to sub-mountain. It is widely distributed in Africa and South America where it is valued as vegetable (Osafo et al., 2016). The plant is popularly known as 'Alo anyasi' in Ibo and 'Atori' in Yoruba tribes of Nigeria. It is an integral part of folkloric medicine in most parts of Africa and South America and is used traditionally to manage various ailments such as fevers, gonorrhea, stomach troubles, lung troubles, parasitic infections, convulsions, constipation, hepatitis and poisoning (Osafo et al., 2016). There has been an increasing research interest on *G.brevis* which has led to the reports of its antioxidant (Dakam et al., 2012), antiplasmodial (Tayo et al., 2015), antiallergic and antiarthritic effects (David et al., 2013). Till date, no report has been made about the antidiarheal effects of this plant. The present study aimed to evaluate the anti-diarrheal potentials of methanol leaf extract of *G. brevis* and its solvent fractions in experimental animal models.

2. MATERIALS AND METHODS

Plant Material

The leaves of *Gyphaea brevis* were collected in July, 2017 from Nsukka local government area of Enugu State, Nigeria. They were identified and given a voucher specimen number of PCG/UNN/0094 by Mr Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka and afterwards deposited in the herbarium.

Drugs, Chemicals, Reagents and Other Materials

The solvents used include analytical grades of; n-hexane, dichloromethane, ethyl acetate and methanol (Sigma Aldrich, Germany), Mueller-Hinton agar (Tm Media, India), 0.5 McFarland turbidity standard (prepared from barium chloride, sulphuric acid and water),

distilled water, dimethylsulphoxide (Qualikems India], loperamide (Belgium), atropine (Livealth, India), gentamicin (Slovenia), castor oil (Bell, Nigeria), deactivated charcoal (locally sourced), oral cannula, needles and syringes.

Preparation of Crude Extract and Fractions

The leaves of *G. brevis* were washed thoroughly with water, air-dried at room temperature for 2 weeks and then pulverized. The powder (1.17kg) was macerated with 95% aqueous methanol for 48 h. The mixture was filtered using filter paper (Whatman No.1) and concentrated using rotary evaporator to obtain the crude extract (CE). The crude extract (10g) was fractionated using n-hexane, ethyl acetate, dichloromethane and methanol in the order of increasing polarities and the fractions obtained were designated as HF, DCMF, EF and MF respectively. They were concentrated, dried and stored in a refrigerator for analysis.

Phytochemical Tests

Preliminary phytochemical tests were carried out on the crude extract and fractions using previously described standard procedures (Harborne, 1973).

Experimental Animals

Adult swiss albino mice (18 - 25g) and adult Wistar albino rats (100-150g) of both sexes were obtained from the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The rodents were housed under standard conditions (25 ± 2^{o}C and a 12-hour light/dark cycle) and were maintained on standard livestock pellets (Guinea feed, Nigeria) with unrestricted access to drinking water. All animal experiments were conducted in compliance with the National Institute of Health Guide for care and use of laboratory animals (Pub No.85-23, revised 1985) and in accordance with the University of Nigeria Ethics committee on the use of laboratory animals, registered by the National Health Research Ethics Committee (NHREC) of Nigeria, with the number; NHREC/05/01/2008B. The study protocol was approved by our institution's Ethics Committee.

Acute Toxicity Test

The acute toxicity (LD_{50}) of the crude extract was estimated in albino mice by the oral route using the method described by Lorke (1983) with little modification. The test was done in 2 phases. The first phase involved the determination of the toxic range. The mice were placed in 3 groups (n=3) and doses (10,100 and 1000mg/kg) of each of the crude extract were administered orally to the mice after overnight fast. The mice were monitored for 24 h for death or any sign of acute intoxication. In the second phase, 3 groups (n= 3) with doses (1600, 2900 and 5000 mg/kg) of the crude extract were orally administered. The animals were observed for lethality and signs of acute intoxication for the next 24 h. The LD_{50} was to be calculated as the geometric mean of the highest non lethal dose and the least toxic dose.

Antidiarrheal investigations

Effect of CE and the Fractions on Castor Oil-Induced Diarrhea in Rats

In this model, diarrhea was induced in the rats by oral administration of 0.5 ml of castor oil using the method described by Amsalu et al (2016). Wistar rats of either sex were fasted for 18 h with free access to water and then randomized into twelve groups (n=5) as follows: Rats in group 1 and 2 received CE (250 and 500mg/kg respectively), group 3 and 4 received HF(250 and 500mg/kg respectively), group 5 and 6 received EF(250 and 500mg/kg respectively), group 7 and 8 received DCMF (250 and 500mg/kg respectively), group 9 and 10 received MF (250 and 500mg/kg respectively). Rats in Group 11 served as the vehicle control and were given 10 % Tween 80 (10ml/kg). Group 12 received loperamide 2 mg/kg as the positive control. The castor oil (0.5 ml) was administered orally 1 h after each dosing. The rats were placed in cages with floor lined with transparent white paper that was changed every hour for a total of 4 h. During the observation period, the number of wet stools was recorded. The percentage of diarrheic inhibition was calculated using the formula below;

$$\%\text{Inhibition} = \frac{\text{mean number of } WF_c - \text{mean number of } WF_t}{\text{mean number of } WF_c} \times 100$$

Where, WF_c = wet feaces in control group and WF_t = wet feaces in treatment group.

Effect of Crude Extract and Fractions on Gastrointestinal Motility

Albino mice (n=60) of either sex were used. The animals were fasted for 18h and randomized into groups as in above. Group 12, however, received atropine (10 mg/kg). The test was carried out using the method described by Amsalu et al. (2016). Briefly, thirty minutes after dosing with the extracts and fractions, the animals were fed orally with 5 % deactivated charcoal (0.5 ml) in mucilage of tragacanth. Thirty minutes later, they were sacrificed by cervical dislocation. The distance travelled by the charcoal plug from the pylorus to the caecum was measured and the percentage inhibition of movement calculated.

$$\% \text{ inhibition} = \frac{D_c - D_t}{D_c} \times 100$$

Where, D_c =mean distance travelled by the charcoal suspension meal in the control group and
D_t = mean distance travelled by the charcoal meal in the treatment group.

Antibacterial assay

Test Organisms

Pure culture of gram positive bacteria, *Enterococcus faecalis* and *Staphylococcus aureus* and gram negative bacteria, *Escherichia coli* and *Klebsiella pneumonia* obtained from the department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka- Nigeria were used in study.

Screening for Antibacterial Activity

The antibacterial activity of the different fractions and crude extract was determined by the agar diffussion method (Boyan et al., 2008). Sterile water (2 ml) placed in test tubes labeled Ec, Ef, Kp and Sa respectively were inoculated with isolated colonies of *E. coli*, *E. faecalis*, *K. pneumonia* and *S. aureus* selected from agar plate culture. The turbidity of the actively growing culture was adjusted to 0.5 McFarland standards ($1.5x10^8$ CFU/ml). About 0.1ml each of the cultured organism was placed in sterile Petri dishes and also labeled as previously. Sterile molten nutrient agar (20 ml) was poured into each of the Petri dishes containing the organisms. The dishes were slightly shook and allowed to gel. A sterile cork borer was used to bore three holes at the center of the seeded agar plates. Five drops each of 10% stock solution (500 mg/ml) of the crude extract was placed in one hole, Gentamicin 10µg/ml as positive control was placed in another and DMSO as vehicle control in the third hole. Sample plates were incubated for 37^0C for 24 h. The procedure was repeated for the different fractions, after which zones of inhibition were measured. The results presented here, are the mean of three replicate testing.

Statistical Analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA; Post hoc- Dunnett comparisons) in a computer–aided software- Graph Pad Prism, version 7.1(Graph Pad Software Inc., San Diego, CA, USA). The results are expressed as mean ± standard error of mean (SEM). The values of the treated groups were compared with those of the control and the difference considered significant at $P< 0.05$.

3. RESULTS

The percentage weight of the crude extract was 6.95% w/w. DCMF, MF, EF and HF yielded 14.2, 31.0, 17.5 and 9.5% w/w respectively. The secondary metabolites present in the extract and fraction are as shown in Table 1. The HF contained the least amount of these compounds.

Table 1 Phytochemical Profile of the leaf extract and fractions of G.brevis

Phytochemical constituents	CE	HF	EF	DCMF	MF
Alkaloids	+++	-	-	+++	+++
Flavonoids	+	-	+++	+++	+
Saponins	+++	-	+++	+	+
Glycosides	+	-	+	-	++
Tannins	+++	-	+++	-	+++
Steroids	+	+	++	+++	+
Terpenoids	+++	+	-	++	-

+++ Very high in concentration, ++ moderately high in concentration and + Low in concentration, - not present.

Oral acute toxicity study

The acute toxicity study indicated that the crude extract of *G. brevis* was relatively safe since there was no mortality at doses up to 5000 mg/kg. Physical and behavioral observations of the experimental mice also revealed no visible signs of overt toxicity. This suggests that the LD_{50} is greater than 5000mg/kg.

Effect of the extract and fractions on Castor oil induced diarrhea

There was a dose-dependent increase in protection from diarrhea induced by castor oil as evidenced by the significant ($p < 0.001$) delay in the onset of diarrhea in most of the treated groups. The groups that received CE (500 mg/kg) and DCMF (250 and 500 mg/kg) had significant ($p<0.01$, $p<0.001$) reduction in the number of wet stool with DCMF (500 mg/kg) offering the highest (94.44%) protection. A moderate but insignificant protection was observed with other fractions (Table 2, Fig 1).

Table 2 Effect of the extract and fractions on Castor Oil Induced Diarrhea in rats

Treatment	Dose (mg/kg)	Mean onset of diarrhea (mins)	Mean number of wet faeces in 4h
CE	250	120.0±3.03***	4.50±2.89
	500	200.0±3.30***	1.00±0.71**
HF	250	90.0±7.07***	5.00±0.60
	500	150.0±3.23***	3.50±0.30
EF	250	150.0±3.44***	4.75±0.85
	500	160.0±3.99***	2.67±0.88
DCMF	250	165.0±3.44***	1.75±0.63**
	500	230.0±3.30	0.25±0.01***
MF	250	110.0±3.21***	3.50±1.26
	500	200.0±3.73***	4.00±1.58
Loperamide	2	240.0±3.08***	0.00 ±0.00***
Tween 80	5ml/kg	39.2±3.72	4.50±0.87

CE, Crude extract, EF, Ethyl acetate fraction, HF, n- Hexane fraction, DCMF, Dichloromethane fraction, MF, Methanol fraction. n = 5 for all groups. **p < 0.01, *** p < 0.001, compared to the vehicle control group.

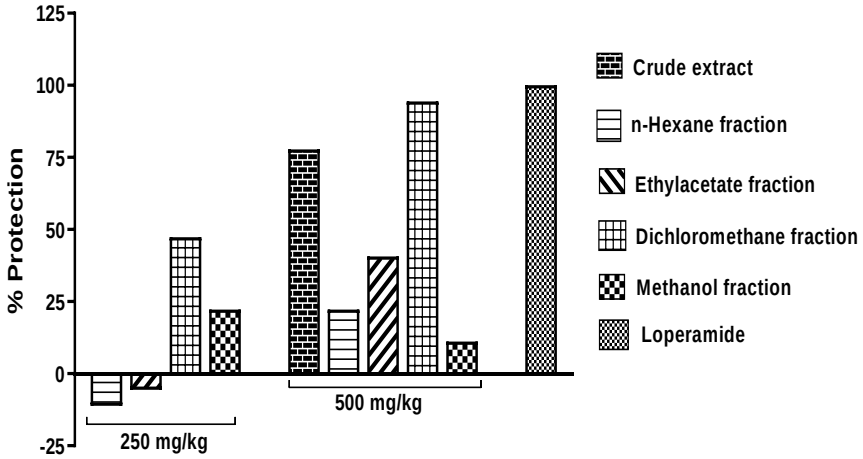

Figure 1 Percentage protection from castor oil induced diarrhea.

Effect of the extract and fractions on gastrointestinal motility

In all the treatment groups, there was a dose-dependent retardation in the propulsive movement of the charcoal meal in the intestine. The MF (250 and 500 mg/kg) significantly ($p<0.01$) caused 75.72%, and 80.12% inhibition respectively on the transit of charcoal meal, which was greater than that of atropine (10 mg/kg) (70.42%). The other fractions evoked significant inhibition only at 500 mg/kg (Figure. 2).

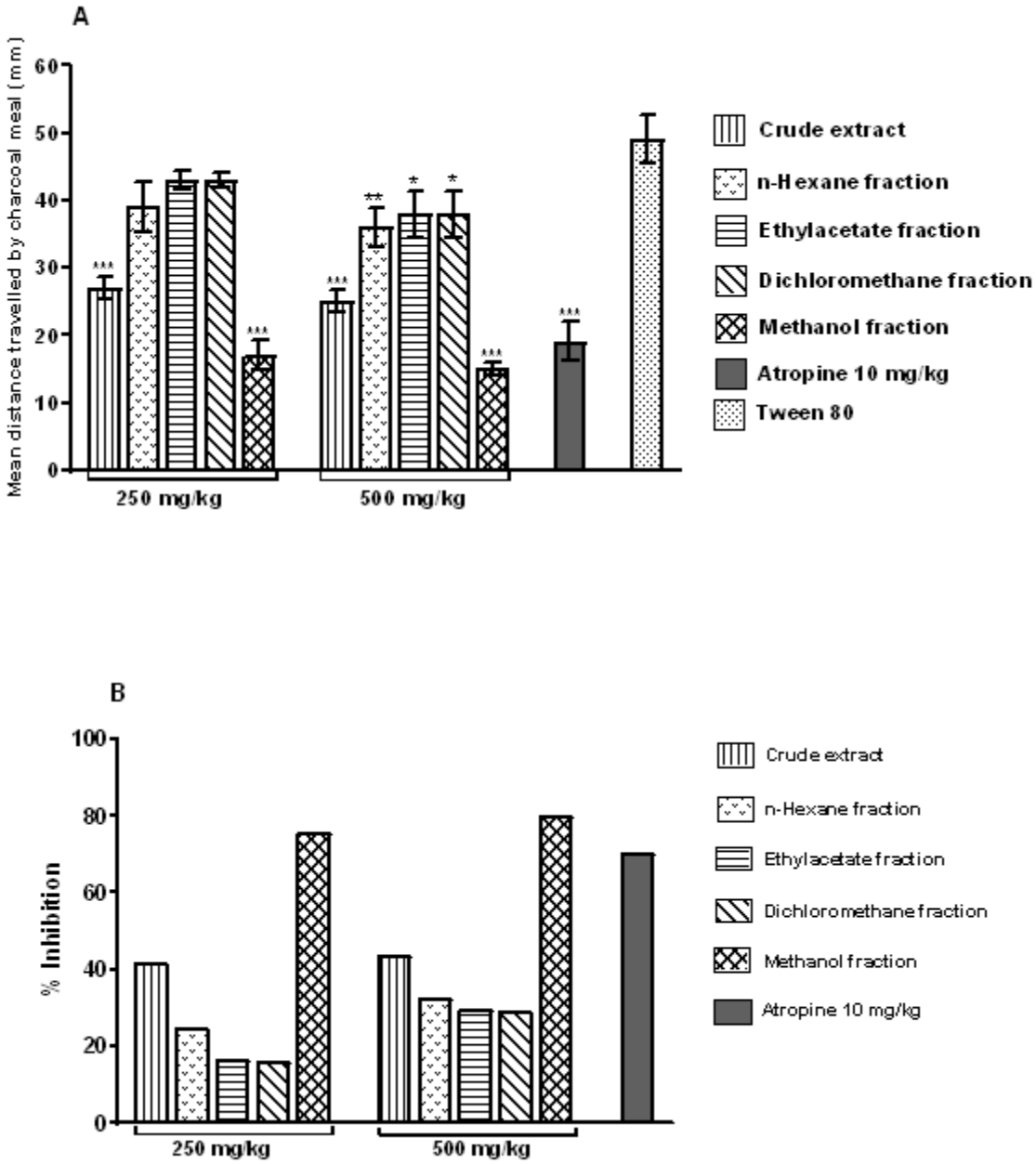

Figure 2 Effects of treatment with methanol extract and fractions on hyperkinetic (propulsive movement) gastro-intestinal tract **(A)** and Percentage inhibition **(B)** of propulsive movement of gastro-intestinal tract.* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Antibacterial Assay

The antimicrobial screening revealed that the extract and fractions did not inhibit the growth of any of the tested organisms after three independent testing (Table 3). Thus it is practically impossible to determine the MIC and MBC of the extract and fractions against the organisms.

Table 3 Preliminary antimicrobial screening of effect of methanol extract and fractions showing inhibition zone diameters (mm)

	Concentration (500mg/ml)						
Test organism	CE	HF	EF	DCMF	MF	GTN	DMSO
Enterococcus faecalis	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00	16.00	0 ± 00
Staphylococcus aureus	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00	26.00	0 ± 00
Escherichia coli	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00	17.00	0 ± 00
Klebsiella pneumonia	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00	15.00	0 ± 00

Negative control : Dimethylsulphoxide, CE, Crude extract, EF, Ethyl acetate fraction, HF, n- Hexane fraction, DCMF, Dichloromethane fraction, MF, Methanol fraction, GTN, Gentamicin. Table represents the mean values of three independent experiment

4. DISCUSSION

The use of plant derived medicines for the treatment of diarrhea is a common practice in many folk medicines. Many people in the developing countries still rely on the treatment system employing medicinal plants. The present study was undertaken to ascertain if the folkloric use of *Glyphaea brevis* in the treatment of diarrhea in south eastern Nigeria has any merit and to speculate the possible active constituents responsible for the activity. Castor oil induced diarrhea and gastrointestinal motility models were used. Also, the antimicrobial activity of the crude extract and the fractions were tested.

Antidiarrhea agents mostly act by decreasing secretion and/or reducing the propulsive movement of gastrointestinal smooth muscles. Castor oil induces diarrhea through a pathophysiological mechanism induced by its active metabolite, ricinoleic acid. Once castor oil is administered orally, it is broken down into ricinoleic acid; a hydroxylated fatty acid by the action of intestinal lipases in the intestinal lumen and considerable amounts of ricinoleic acid is absorbed in the intestine. Ricinoleic acid then mediates its action by binding with EP_3 prostanoid receptors on smooth muscle of the intestine (Tunaru et al., 2012). More precisely, castor oil elevates the biosynthesis of prostanglandin which results in the irritation and inflammation of the intestinal mucosa to stimulate motility (increased propulsive movement) and secretion (Abu et al., 2013). In addition, it forms ricinoleate salts with sodium and potassium in the lumen of the intestine and these salts inhibit sodium-potassium ATPase and increase permeability of the intestinal epithelium, which in turn produces a cytotoxicity effect on intestinal absorptive cells (Komal et al., 2012). With the death of absorptive cells, there will be a decrease in water and electrolyte re-absorption and hence increase in fluid accumulation in the gut. Therefore, the use of castor oil as diarrhea inducer for this model is plausible as it mimics the pathophysiological process by not only increasing gastrointestinal motility but also increasing intestinal secretion. Based on our study, the antidiarrheal activity of the most active fraction DCMF may be mediated by antisecretory mechanisms, as well as inhibition of gastrointesinal motility.

Tannins, flavonoids, alkaloids, sterols/terpenes (Mazumder et al., 2019), saponins and reducing sugars (Abu et al., 2013) have been found to be responsible for antidiarrheal effects of medicinal plants. Terpenoids such as abietic acid, steroids like physosterols and flavonoids have been shown to inhibit production of prostaglandin E2 which is known to play a crucial role in the stimulation of intestinal secretions (Igor, 2015; Amsalu et al., 2016). In addition, flavonoids present antioxidant properties which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism (Lei et al., 2016) and consequently prostanglandin synthesis. The abundance of terpenoids and flavonoids in DCMF and CE may be responsible for the inhibition of diarrhea normally caused by castor oil. The active metabolite of castor oil (ricinoleic acid) might also activate the nitric oxide pathway and induce nitric oxide (NO) dependent gut secretion (Mekonnen et al., 2019). A growing body of evidence indicates that phytochemical constituents such as flavonoids (Filomena et al., 2019), terpenoids (Mekonnen et al., 2019) and alkaloids (Martha et al., 2018) are implicated in the attenuation of NO synthesis. Thus, the pronounced inhibition of castor oil mediated intestinal fluid secretion found with DCMF could be linked to the presence of flavonoids, terpenoids and alkaloids that might have increased the reabsorption of electrolytes and water by hindering castor oil mediated NO synthesis as NO has been reported to play an important role in castor oil-induced diarrhea (Hu et al., 2009).

Based on our results, a significant inhibition in the transit of charcoal meal along the gastrointestinal tract which depicts decrease in gastrointestinal motility occurred only with the methanol fraction at all the tested doses. Studies on the functional role

of tannins show that they inhibit gastrointestinal movement by reducing the intracellular Calcium ions inward current or by the activation of calcium pumping system as well as forming tannates which make the intestinal mucosa more resistant to movement and hence reduction in peristaltic movement (Belemtougri et al., 2006). Alkaloids have also been demonstrated to have inhibitory effect on gastrointestinal motility (Ambreen et al., 2018). The presence of alkaloids and tannins in this fraction may therefore account for its potent inhibitory effect on gastrointestinal motility.

Although the phytochemical constituents that are responsible for the antidiarrheal effect are yet to be identified, the amount of phytochemical constituents that are responsible for impeding the gastrointestinal motility such as tannins (Belemtougri et al., 2006) and alkaloids (Ambreen et al., 2018) appear to increase with dose and may be the possible reason why significant reduction in the propulsive movement of gastrointestinal tract was observed at a higher dose with the methanol fraction. However, from the study, this fraction even though caused significant delay in the onset of diarrhea, did not elicit significant inhibition of diarrhea induced by castor oil. This might be due to the absence/low concentration of some secondary metabolites such as terpenoids, steroids (Amsalu et al., 2016) and flavonoids (Igor, 2015), because flavonoids have been shown to inhibit hydroelectrolytic secretions which are altered in diarrheic condition (Bizuneh et al., 2018) while terpenoids and steroids (physosterols) also inhibit prostaglandin E2 induced fluid secretion in the intestine (Igor, 2015).

Neither the fractions nor the crude extract showed antimicrobial activity, thus they cannot be used in the treatment of infective diarrhea caused by the tested organisms. Meanwhile, (Dickson et al., 2011) reported that the ethanolic extract of the leaf of this plant inhibited the growth of some gram positive bacteria but still ineffective in all the gram negatives tested. The result is in agreement with our study in which there was no inhibition of the growth of gram negatives. The different solvents used in the extraction may be responsible for the different result obtained in the gram positive sensitivity result. According to Lorke (1983), any substance that is not toxic at 5000mg/kg is considered relatively safe. The extract may therefore, be considered safe since no death was recorded at 5000mg/kg and there were no behavioural alterations in the animals.

5. CONCLUSION

The present study revealed that the leaves of *G. brevis* possess antidiarrheal activity with the methanol and dichloromethane fractions as the most active fractions. The antidiarrheal activities could be attributed to the presence of some bioactive agents, including, tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids that may acting individually or collectively. These results, therefore validates the folkloric use of this plant extract in the treatment of diarrhea. Further studies that aim to isolate the active principle(s) and elucidate the possible mechanism(s) of action using the two fractions (DCMF and MF) are underway.

Competing Interest

There are no conflicts of interest in the study.

Authors' Contribution

MNO designed the study and performed some of the experiments, PAI, ONI, IEP, and PEU performed the experiments, MOA did the extraction and fractionation, while PAA prepared the manuscript for publication.

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Data and materials availability:

All data associated with this study are present in the paper.

REFERENCE

1. Abu M.T, Muhammad E.U, Asharf U.C et.al. *In Vivo* Antidiarrheal and Cytotoxic Potential of Different Fractions of *Pandanus Foetidus* Leaves. American Journal of Biomedical Sciences. 2013:(3)5:208-16.
2. Ambreen A, Khalid HJ. Dual Mechanism of Anti-muscarinic and Ca^{2++} antagonistic activities to validate the folkloric uses of *Cyperus niveus* Retz. As antispasmodic and antidiarrheal. Journal of Ethnopharmacology. 2018: 213: 138-48.
3. Amsalu D, Ephrem E, Workineh S. Evaluation of the anti-diarrheal activity of the leaf extract of *Croton macrostachyus* Hoccsht. ex Del. (Euphorbiaceae) in mice model. 2016: 16:379.
4. Belemtougri RG, Constantin B, Cognard C, et al. Effects of *Psidium juajava*L.(Myrtaceae) and *Diospyros mespiliformis* L.(Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture. Journal of Zhejiang University Science. 2006: 7:56-63
5. Bizuneh W, Reta R, Tibebu A, and Moa M. Medicinal Plants Used for Treatment of Diarrhoeal Related Diseases in Ethiopia. Evidence-Based Complementary and Alternative Medicine. 2018:3:1-20.
6. Boyan B, James H, Juducael P. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. Journal of Antimicrobial Chemotherapy. 2008:61(6):1295-1301
7. Dakam, W, Oben, J.E & Ngoyang J. Y. Antioxidant activity and extractability of phenolic compounds from medicinal plants: A Study of *Glyphaea brevis* Sreng.(Monach). Pharmacology Online 2012: (3): 708-18
8. David D.O, Newman O, Regina E.A. Antiallergic and antiarthritic effects of stem bark extract of *Glyphaea brevis* (Spreng) Monachino (Tiliaceae) in murine models. ISRN Pharmacology. 2013: 1-13
9. Dickson RA, Ekuadzi E et.al. Antibacterial,anti-inflammatory and antioxidant effects of the leaves and stem bark of *Glyphaea brevis*(Spreng) Monachino (Tiliaceae): A comparative study. Pharmacognosy Research. 2011:3(3): 166-72.
10. Filomena P, Luca F , Ian H et al. Naturally occurring sesquiterpene lactones and their semi-synthetic derivatives modulate PGE2 levels by decreasing COX2 activity and expression. Heliyon. 2019:*5*(*3*):e01366
11. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall, New York. 1973. pp 267-70
12. Hu Jing, Goa W, et.al. Antidiarrheal and intestinal modulatory activities of Wei-Chang-An-Wan extract. Journal of ethnopharmacology. 2009: 125(3): 450-5
13. Igor RP, Flavia DF et.al. *Cissampelos sympodialis* Eichl.(Menispermaceae), a medicinal plant, presents antimotility and antidiarrheal activity *in vivo*. BMC Complementary and Alternative Medicine 2015:(15):253
14. Komal, Kumar S, Rana A C. Herbal Approaches for Antidiarrhea: A Review. International Resource Journal of Pharmacy. 2012:(1)4: 31-8
15. Lei C, Hui T et.al. Modifications of dietary flavonoids towards improved bioactivity: An update on the structure activity relationship. Critical Review in Food Science and Nutrition. 2016:58:4
16. Lorke D. A new approach to practical acute toxicity testing. Archive of Toxicology. 1983: 53:275-87.
17. Martha A M, Mary A M and Pigweh I A. Phytochemical Screening and Anti-diarrheal Activity of the Pul and Seed Extracts of *Ziziphus mauritiana* in Rats. International Journal of Biochemistry Research and Review. 2018: 24(3):1-9
18. Mazumder K, Dey ZK, Dey S, et.al. Phytochemical screening and evaluation of antidiarrheal mode of action of leaves extracts of *Paederia foetida*. Linn. International Journal of Complementary and Alternative Medicine 2018:(5)11:304-9
19. Mekonnen S, Neegussie B, Tigist G. Evaluation of the antisplasmodic and antisecretory activities of the 80% Methanol Extract of *Verbena officinalis* L: Evidence From In Vivo Antidiarrheal Study. Journal of Evidence-Based Integrative Medicine. 2019:24:1-9
20. Osadebe P.O, Chika C.A, Matthias O.A. Antimotility effects of extract and fractions of Eastern Nigeria mistletoe (*Loranthus micranthus* Linn). Asian Pacific Journal of Tropical Medicine. 2012: (7)5: 556-60.
21. Osafo N and Yaw D.B. *Glyphaea brevis* (Spreng.) Monach. A Review of the Ethno-Medical, Phytochemical and Pharmaceutical investigations. British Journal of Pharmaceutical Research. 2016: 12(2): 1-18
22. Tayo M.A, Sani I & Andrew J N. Evaluation of *in-vivo* antimalarial activity of methanol leaf extract of *Glyphaea brevis* in *Plasmodium berghei*-infected mice. Tropical Journal of Pharmaceutical Research. 2015: (10)14: 1837-42
23. Tunaru S, Althoff TF, Nusing M et al. Castor oil induces laxation and uterus contraction via ricinoleic acid activating prostaglandin EP3 receptors. Proceedings of the national academy of sciences. 2012: 109:9179-84.
24. United Nations Children's Fund. One is Too Many: Ending Child Deaths from Pneumonia and Diarrhoea. 2016. https://www.unicef.org/publications/index_93020.html.
25. World Health Organization. Diarrheal Disease, 2017. http://www.who.int/mediacentre/factsheets/fs330/en/.
26. World Health Organization. Health topics on Diarrhea, 2016. http://www.who.int/topics/diarrhoea/en/.